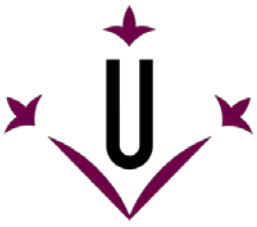

Intra-specific association between carbon isotope composition and productivity in woody plants: a meta-analysis

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Common abbreviations:

WUE_i, intrinsic water- use efficiency, $\delta^{13}C$, Carbon isotope composition, $\Delta^{13}C$, Carbon isotope discrimination, *Gr*, global estimated correlation coefficient, g_s, stomatal conductance, *A*, assimilation rate, *Q*, statistic of heterogeneity, I^2, statistic of between-studies heterogeneity, *QM*, test statistic for the Wald-type test of model coefficients (moderators), R^2, amount of heterogeneity accounted for by the moderators included in mixed effect models.

Highlights

- Water use efficiency and growth show positive intra-specific correlations in trees
- Association increases from Mediterranean to subtropical, temperate and boreal biomes
- Positive correlations dominate in conifers and shrubs, but not in broadleaves
- Correlation is higher for seedlings, and using leaf $\delta^{13}C$, than for adults and wood
- The strongest genetic associations appear under controlled and optimal conditions

ABSTRACT

The study of intra-specific variations in growth and plant physiological response to drought is crucial to understand the potential for plant adaptation to global change. Carbon isotope composition ($\delta^{13}C$) in plant tissues offers an integrated measure of intrinsic water use efficiency (WUE_i). The intra-specific association between $\delta^{13}C$ and productivity has been extensively studied in herbaceous crops, but species-specific information on woody plants is still limited and has so far provided contradictory results. In this work we explored the general patterns of the relationship between $\delta^{13}C$ and growth traits (height, diameter and biomass) using a meta-analysis. We compiled information from 49 articles, including 176 studies performed on 34 species from 16 genera. We found a positive global intra-specific correlation between $\delta^{13}C$ and growth (Gr= 0.28, P<0.0001), stronger for biomass than for height, and non-significant for diameter. The extent of this intra-specific association increased from Mediterranean to subtropical, temperate and boreal biomes, i.e. from water-limited to energy-limited environments. Conifers and shrubs, but not broadleaves, showed consistent positive intra-specific correlations. The meta-analysis also revealed that the relationship between $\delta^{13}C$ and growth is better characterized at juvenile stages, under near-optimal and controlled conditions, and by analyzing $\delta^{13}C$ in leaves rather than in wood.

Key words: *water-use efficiency; growth; height; diameter; genetic variation; biomass*

1 Introduction

Productivity in over 40% of the vegetated land area is limited by water availability, while another 33% experiences water limitations due to constraints associated with cold temperatures or inaccessibility of frozen water [1]. Hence, there is a clear dependence of plant productivity on water use (i.e. amount of water evapotranspired) around the globe [2]. Foresters are especially interested in wood production, an outcome of secondary growth from vascular cambium leading to the radial growth of tree stems, branches and roots. In addition to the variability caused by the environment, woody species also show genetic variation in their growth characteristics due to differences at the population, family or clonal levels [3-5]. Under future climate change scenarios, the importance of water scarcity as a factor limiting plant growth and terrestrial ecosystem productivity might increase worldwide, particularly in arid and semi-arid regions ([6], but see [7]). In this context, the study of intra-specific variability in growth and associated physiological responses to drought is crucial to understand the potential for adaptation of forest trees to climate change [3,8].

Carbon isotope composition ($\delta^{13}C$) in plant tissues offers an integrated measure of the ratio between chloroplastic and atmospheric CO_2 concentrations (C_c/C_a), and hence can be used to estimate the ratio between net CO_2 assimilation rate (A) and stomatal conductance for water vapour (g_s), i.e. intrinsic water use efficiency (WUE_i) after making certain assumptions about mesophyll conductance and post-photosynthetic fractionations [9]. Plant productivity and WUE_i are correlated at multiple levels, but the nature of this relationship depends mainly on the leading term in the quotient A/g_s; 1) when higher WUE_i is related to an increase in photosynthesis, then positive relationships with growth are expected; 2) conversely, if higher WUE_i implies a tighter stomatal control, it tends to be inversely correlated with growth [10]. The first case is exemplified by genotypes with higher photosynthetic potential, showing a higher WUE_i than low photosynthesis, less productive ones [11,12]. In the second case, high WUE_i results from low water use and consequently, is linked to lower productivity [13,14].

The use of carbon stable isotopes to characterize plant genetic variability in WUE_i is generally preferred over conventional gas exchange methods (i.e. infra-red gas analysers) because it provides a less disturbing, more integrated record of the ratio A/g_s over the period in which the plant tissue was formed [10,15]. Nonetheless, there are several aspects that introduce uncertainty when analysing the relationship between WUE_i and growth other than the

variability in A/g_s. For instance, A determines carbon inputs, but growth is only one of the many possible fates of assimilates [16]. Indeed, increasing A does not always lead to a concomitant increase in growth [17,18]. Broadly speaking, fixed carbon can be allocated to either above- or below-ground organs, and the proportional allocation to roots relative to that in shoots depends upon the degree of limitation experienced by aboveground resources (e.g. low light intensity produces etiolation) relative to the limitation from soil resources (e.g. water or nutrient scarcity enhances root growth [19,20]). Emission of volatile organic compounds or the non-linear increase of respiration rates with tree size [21,22] further complicates the theoretical relationship between *WUE*i and aboveground growth. Therefore, although this work focusses on the association between *WUE*i and aboveground growth (a feature of interest for foresters and, hence, a prevalent topic in the literature), a different picture could potentially emerge if whole plant growth had been considered instead.

The intra-specific association between WUE_i and productivity has been extensively studied in herbaceous crops. In general, the most productive genotypes are water spenders and, thus, show lower WUE_i, although this relationship can be inverted, particularly under drought-stress conditions. Contrasting to crops, information on woody species is still limited and has provided opposing results so far [14,23-28]. In this regard, the general patterns of the intra-specific relationship between WUE_i and tree growth have not yet been described. In the present work, we have carried out a meta-analysis on the association between $\delta^{13}C$ and growth traits (height, radial growth and biomass) in woody species, with a focus on forest trees. To this end, potential differences in this association among functional groups, terrestrial biomes or environmental conditions for growth have been assessed. We hypothesize that $\delta^{13}C$-derived WUE_i and growth would show positive intra-specific associations under non-limiting water conditions and for fast-growing, highly productive species, when changes in A would play a major role controlling WUE_i. Conversely, the opposite situation would prevail under drought stress and for less productive species, since growth potential may trade-off with drought resistance and, therefore, g_s is expected to control WUE_i.

2 Materials and Methods

2.1 Data Collection

We searched published articles from the databases "Web of Science", "Scopus", "Science Direct" and "Google Scholar" using the terms: ("genotypic variation" OR "genetic variation" OR "genetic differentiation" OR "genetics" OR "intraspecific variability" OR "population differences" OR "Genotype" OR "water use efficiency" OR "Carbon isotopic discrimination" OR "carbon isotope composition" OR "genetics of water- use efficiency" OR "genetic variation in water- use efficiency" OR "water deficit condition") AND ("relation to tree growth" OR "growth" OR "biomass production" OR "productivity" OR "height" OR "diameter" OR "volume") AND ("tree" OR "seedling" OR "forest"). References from each article were also systematically examined for suitable papers (backward search), which were then incorporated into the bibliographic database. We selected those papers reporting intra-specific associations (Pearson correlation coefficients or genetic correlations) between carbon isotopes and growth traits based on common-garden experiments (either in the field or in pots). We also included those studies in which the correlation coefficients between $\delta^{13}C$ and growth were not explicitly provided but, instead, useful data (genotype means) for $\delta^{13}C$ and growth could be retrieved from tables or graphs. In all cases, we only considered associations stemming from a minimum sample size of five genetic entities.

We ended up with 49 articles in our dataset, published from 1994 to 2015, and one unpublished dataset from a congress proceeding. When correlations were available in the same paper for more than one species, type of genetic material, experimental condition, trial site or treatment, we counted them as independent studies. If correlation involving $\delta^{13}C$ was reported for two or more growth parameters (height, diameter or biomass) in the same paper, we also considered each correlation as an individual study in the dataset. Nevertheless, we discarded clearly redundant correlations (e.g. with the same growth trait over time, or for the same trial accounting for different genetic levels, e.g. populations and families). This resulted in a total of 176 studies of 34 species in 16 genera. In those cases reporting correlations between growth and carbon isotope discrimination ($\Delta^{13}C$), we simply changed the sign of the correlation coefficient, as $\Delta^{13}C$ values are almost mirror images of $\delta^{13}C$ records (provided the isotope composition of the air is constant across observations). Individual correlation coefficient (r) and sample size (i.e. number of genetic entities, n) were then tabulated for the

meta-analysis (Appendix A, Tables A.1).

2.2 Data analyses

We conducted all the analyses using the *metafor* statistical package [29] in the R statistical environment (R 3.0.2, R Development Core Team, 2013). The meta-analytical model was fitted with the function rma.uni, and is a special case of the general linear (mixed-effects) model. The inputs for the model are assumed to be unbiased and normally distributed [29]. Therefore, we applied the 'Fisher' Z-transformation to calculate an effect size for each individual study according to a normal distribution: $Z = 0.5\ log\ [(1+r)/(1-r)]$, with the corresponding asymptotic variance as a function of sample size: $vi=1/(n-3)$ [29,30]. To choose between fixed- or random-effects models, we considered the significance of the statistic *Q* (a *Q* test, seee [31]). The *Q* test determines the presence of heterogeneity, but it does not give a quantitative measure of the extent of such heterogeneity. Therefore, we also considered the I^2 statistic, as a quantitative measure of true heterogeneity to the total variance, that is, the proportion of the between-studies variability with respect to the total variability [32]. The meta-analysis provides an inference of the average true effect in the population of studies, in our case an estimated global correlation coefficient effect size (*Gr*), which has a normal distribution, equivalent to that of the individual *Z*-transformed effect sizes. Therefore, unlike correlation coefficients, *Gr* ranges between $-\infty$ and $+\infty$. Z values can be easily reverted to a correlation coeffient (range: -1 to +1) using the inverted Fisher transformation. However, since the value of *Z* and *r* do not differ substantially with $r<0.50$, and this was generally the case, for simplicity only the *Gr* coefficients were displayed throughout the manuscript.

Firstly, a general meta-analysis was calculated for the whole dataset ($N=176$) of normalized (Z-transformed) effect sizes. Subsequently, a number of moderators (i.e. variables potentially explaining the hetereogeneity in the dataset) were tested using mixed-effects models [29]. These models provide an omnibus (Wald-type) test of the significance of model coefficients (*QM* statistic), informing on the amount of heterogeneity accounted for by the moderators included in the model (R^2), as well as a test for the significance of differences among the different levels of the moderator. For those moderators showing a significant effect in the omnibus test, we then performed separate meta-analyses for each level of the variable. The moderators tested included both methodological and biological factors (see Table 1 for a list of moderators and their levels). As a general rule, we defined moderator levels according to the principle of parsimony, attempting to include the most relevant sources of variability,

while ensuring a meaningful sample size within each level. For example, growth traits are determined and reported in miscellaneous forms in the literature (Table 1), but total height, diameter and stem biomass are the most frequent. Hence, growth traits were finally combined into three main groups: 'height', 'diameter' and 'biomass'. Similarly, 'leaves' and 'branchlets', among plant parts, and 'greenhouse' and 'nursery', were grouped together as 'green parts' and 'controlled environments', respectively. About one fourth of the studies investigated the effect of $\delta^{13}C$ on tree growth in two water treatments, irrigated and drought, and for these cases we tested water treatment as a potential moderator. The rest of studies were classified into two subsets, 'optimal' and 'suboptimal' testing conditions, according to the description given by the authors. We also tested a combination of these two factors, considering drought and irrigated treatments as suboptimal and optimal environments, respectively. A comprehensive list of studies, including the raw correlation values, and the assigned values for each moderator, is given in Appendix A (Tables A.1 and A.2).

Finally, a potential publication bias (i.e. the tendency for non-significant results to remain unpublished) was tested with a regression test for funnel plot asymmetry [33] and the robustness of the overall effect tested with the fail-safe number [34]. The regression test assumes that if studies with small correlation coefficients remain unpublished this would result in an asymmetric funnel plot, and tests this by examining the relationship between observed values and the corresponding sampling variances, sample sizes or standard errors. The fail-safe number estimates the number of non-significant studies needed to make the current *Gr* non significant, and is usually compared against a reference value of $5n + 10$ [34].

3 Results

3.1 General trends in the relationship between $\delta^{13}C$ and plant growth traits

We observed a general positive correlation between $\delta^{13}C$ and growth, combining the data from all species and growth traits (*Gr*= 0.28, *P*<0.0001; 95% Confidence Interval: 0.18-0.38). 126 out of the 176 studies showed a positive trend, and about one third of the total (49) had significant positive correlations (p<0.05). On the contrary, only 7 studies showed a negative and significant correlation between $\delta^{13}C$ and growth.

Despite the significant general trend, we observed a high level of variability between studies (Figure 1). The *Q* statistic for heterogeneity was significant (*P*<0.0001) for the whole dataset and also for most of the subsets tested, and the I^2 statistic showed in most cases medium to high values (Appendix A, Table A.5). The only exceptions were the subsets 'Total dry mass', 'Volume' and 'basal area index', among growth traits, and the experimental set-up 'nursery', which did not show significant heterogeneity, and showed low I^2 values (Table A.5). Based on the heterogeneity test, we used fixed-effects model to calculate the *Gr* for these two subsets, and random-effects model for the rest of subsets.

We did not find evidence for publication bias in our dataset. The regression test for funnel plot assymetry did not show any significant correlations with the predictors tested (sample size, sampling variance and standard error), and the fail-to-safe number for the whole dataset was very large (9007 studies), i.e. over ten-fold larger than the reference value (5 × 176 + 10 = 890).

3.2 Main factors affecting the relationship between $\delta^{13}C$ and growth

As shown in Table 1, the test for moderators revealed strong differences among growth traits, explaining up to 25.8% of the total variability, considering the seven original levels, and 18.5% after combining the different variables into three large groups (height, diameter and biomass). Nevertheless, taxonomic and ecological differences explained most of the between-studies variability, with species, genus and biome explaining 85.0%, 73.7% and 19.5% of the heterogeneity, respectively (Table 1). The test for moderators also revealed strongly significant effects (*P*<0.001) of ontogenic stage (17.4% of the heterogeneity). Other moderators showed weaker but significant effects, such as experimental set-up, life-form,

environment or plant part (Table 1). On the contrary, type of genetic material, type of correlation and leaf habit did not accounted for a significant amount of heterogeneity. The combination of the most significant experimental-related moderators (ontogenic stage, plant part, experimental set-up and environmental conditions) explained 21.7% of the total heterogeneity (*QM*= 28, d.f.=8, $p = 0.0005$).

3.2.1 Growth traits, ontogenic stage and plant parts

Among the different growth traits, total height, total stem biomass and total volume showed strongly significant ($p<0.001$) positive associations with $\delta^{13}C$, whereas total diameter and dry mass showed a weak, but still significant positive association (Table 2). Conversely, basal area increment showed a significant negative correlation ($p=0.033$), although based on only four studies. Neither height increment nor diameter increment were significantly associated with $\delta^{13}C$. After combining the different growth traits into the three main groups (height, diameter and biomass), the same overall trends were observed. Whereas height- and biomass-related traits maintained a strong positive association with $\delta^{13}C$ (*Gr* = 0.28 and 0.68 respectively), diameter-related variables did not show a common significant response (Table 2). Among the 112 studies reporting correlations between $\delta^{13}C$ and height or biomass, about one third showed a significant positive association (19 for biomass and 20 for height). On the contrary, we did not find significant negative correlations with biomass, with only three cases for height (involving annual height growth in *Juglans regia* [23] and total height in *Pinus halepensis* [14]).

Individual correlations between diameter and $\delta^{13}C$ showed positive trends in 38 out of 64 studies, but only 10 showed a significant positive association, against 4 cases with negative significant correlations. We observed a high positive Z-transformed effect size on the correlation between diameter and $\delta^{13}C$ for *Salix* clones [27], and for families of two boreal conifers, *Picea mariana* [24] and *Larix occidentalis* [28]. On the contrary, strong negative effects were observed for diameter increment in the Mediterranean *J. regia* [23], and total diameter in temperate *Eucalyptus globulus* [35] and the subtropical shrub *Acacia senegal* [13].

We also observed significant ontogenetic effects, such that *Gr* was weaker in adult trees (0.26) than in seedlings (0.44), and non-significant in saplings (p = 0.51; Table 2). Non-significant global correlation in saplings was driven by a comparable proportion of negative

and positive effects (18 and 22, respectively), and a similar amount of significant correlations in both senses (6 negative, 5 positive). Regarding the material used for the determination of $\delta^{13}C$, studies performed on green tissues (leaves and branchlets) showed a strongly significant positive correlation (*Gr*=0.36), whereas global effects on wood were weak and only marginally significant (Table 2). 43 out of the 137 studies using green tissues $\delta^{13}C$ showed a positive significant association with growth traits, against only 5 cases showing significant negative effects. Conversely, among the 39 studies using wood $\delta^{13}C$, we found significant positive and negative effects in 6 and 2 studies, respectively.

Considering the differential response of each growth trait, studies on saplings showed a negative (though non-significant) response to $\delta^{13}C$ in height and diameter, contrasting to the strongly significant positive association between $\delta^{13}C$ and biomass ($p<0.001$, Figure 2a). On the contrary, studies with seedlings and adult trees showed significant positive associations with all traits (Figure 2a). Growth traits also differed in their response to $\delta^{13}C$ measured in green tissues or wood (Figure 2b). Whereas the association between $\delta^{13}C$ and biomass was positive and significant regardless of the plant part used, height only showed a significant association with $\delta^{13}C$ when measured in green tissues. Diameter was not significantly correlated with either green tissue or wood $\delta^{13}C$.

3.2.2 Life form and biomes

Plant growth was positively associated with $\delta^{13}C$ in shrubs (*Gr* =0.57) and conifers (*Gr* = 0.34; Table 2), but not in broadleaves (*p* = 0.25). For shrubs, the strongest positive associations were found in the subtropical *Prosopis glandulosa* [36] and among temperate *Salix* clones [27], and only one significant negative association was found in *A. senegal* [13]. Similarly, for conifers we only found one significant negative association in *P. halepensis* [14] against 28 out of 102 cases showing significant positive correlations. In general, boreal conifers showed the highest correlations, particularly strong for *Picea mariana* and *Larix occidentalis* under field conditions [37,38], but we also found strong positive associations in other biomes, e.g. for the temperate *Pinus contorta* [39], in a set of tropical pine clones [40] or in the Mediterranean *Pinus pinaster* [41]. Despite the lack of an overall trend among broadleaves, we still found a larger amount of positive significant associations (13 out of 57) than negative ones (5). The strongest positive effect was found for *Castanea sativa* [25] and

the subtropical tree *Faidherbia albida* [42], contrasting with the aforementioned negative effects in *J. regia* and *E. globulus* [23,35].

Among the different biomes, the *Gr* between $\delta^{13}C$ and growth was positive and strongly significant for subtropical, temperate and boreal species (0.20, 0.33 and 0.74 respectively), whereas we did not find a significant global trend within the Mediterranean biome (Table 3). For Mediterranean species, 26 out of 37 cases showed non-significant effects. Significant positive effects were found in 7 studies, six of them conducted in *Pinus pinaster* (e.g. [10,41,43]), and one for *Quercus suber* [44], and only 4 cases showed significant negative effects, in *J. regia* and *P. halepensis* [14,23].

Looking at the response of the different growth traits across biomes, height followed the same global pattern, showing a significant positive association in all biomes, except the Mediterranean (Figure 3a). On the other hand, whereas diameter was only significantly associated with $\delta^{13}C$ in the subtropical and boreal biomes, biomass was positively associated with $\delta^{13}C$ in all biomes (Figure 3a). The distinction among biomes was also affected by the testing environmental conditions, showing larger differences under suboptimal than under optimal conditions (Figure 3b). All combinations of biome and environmental conditions showed positive and significant associations between $\delta^{13}C$ and growth, except for the Mediterranean biome, which showed opposite sign in optimal and suboptimal conditions, although not significant (Figure 3b).

3.2.3 Experimental set up and environmental conditions

Plant growth was positively associated with $\delta^{13}C$ regardless of the experimental set-up, but the association was weaker in the field (*Gr*=0.17) than under controlled conditions (greenhouse and nursery; *Gr* = 0.56, Table 4). Regarding environmental conditions, both irrigated and drought treatments showed a significant positive correlation between $\delta^{13}C$ and drought (*Gr*=0.63 and 0.61, respectively, Table 4). We found significant positive effects in 8 cases for droughted and 7 cases for irrigated. For the rest of studies, where a drought treatment was not applied, only those considered to be performed under near-optimal conditions resulted in a significant positive global effect (*Gr*=0.28). Within the optimal subset, significant positive correlations were clearly dominant (23 out of 88 cases), against

only three cases showing significant negative correlations (in *P. halepensis* [14] and families of hybrid *Populus* [45]). Conversely, among the 49 studies performed under suboptimal conditions, we found a lower disproportion between positive and negative significant correlations (11 and 4, respectively). The positive and significant trend found in optimal conditions was maintained after combining optimal and irrigated subsets (*Gr*=0.34), whereas the combination of suboptimal and drought subsets still showed non-significant global effects (Table 4). Comparing different experimental set-ups, we found that the effect of environmental conditions was maximized in field trials, as compared to more controlled conditions, such as greenhouse or nursery (Figure 4). Whereas under controlled conditions we did not find differences between irrigated, drought and optimal environments (none of the controlled studies were classified as suboptimal), field studies only showed a positive significant trend under irrigated and optimal environments (Figure 4).

4 Discussion

4.1 Positive intra-specific association between WUE_i and growth in woody plants

Globally, $\delta^{13}C$ and growth showed an intra-specific positive correlation in woody plants. Among growth traits, biomass exhibited the strongest relationship with $\delta^{13}C$, and also the most consistent one, with fairly homogeneous results across studies (I^2=26.3). Indeed, none of the studies included in this meta-analysis showed a significant negative correlation between biomass and $\delta^{13}C$. The correlation between $\delta^{13}C$ and height was also strongly significant, but weaker and with higher inter-studies variability. On the contrary, diameter did not show a significant general association with $\delta^{13}C$. These results suggest that biomass, as the most integrative measure of plant growth, may also be the most directly linked to physiological processses determining WUE_i. Several studies have pointed out that height is particularly sensitive to site-specific growing constraints, which could hinder the detection of intra-specific associations between this trait and $\delta^{13}C$. For example, a strong effect of nutrient and water availability on height has been reported in a wide range of growing conditions, from seedlings under controlled conditions [46] to adult trees in the field [47,48]. On the other hand, diameter is usually considered as the growth trait most sensitive to environmental and stand conditions [49], and this might be partly responsible for the contrasting relationships with $\delta^{13}C$ observed in this study.

Seedlings showed the strongest correlation between growth traits and $\delta^{13}C$, and this could be explained by the closer link between leaf-level processes such as WUE_i and whole-tree physiology in young individuals, as compared to older trees [2,22]. The ontogenic stage effect on $\delta^{13}C$ has been observed in several studies. For instance, Marshall and Monserud [50] found that inter-tree variability in $\delta^{13}C$ for ponderosa pine, western white pine and douglas-fir decreased from seedling to sapling and adult stages. Duquesnay et al. [51] also showed greater differences in $\delta^{13}C$ among young individuals of *Fagus sylvatica* than in mature stands. Considering the strong effect of ontogenic stage on the association between $\delta^{13}C$ and growth, it might be argued that the observed differences among growth traits may be partly due to an unbalanced distribution of studies. Most studies on biomass were perfomed at the seedling stage (85%), and none in adult trees, whereas for height we found 54% in seedlings and 26% in adult trees, and a similar number of studies reported correlations with diameter for

seedlings, saplings and adult individuals (see Appendix A, Table A.3). Nevertheless, the larger magnitude of the correlations for biomass in both seedlings and saplings (see Figure 2) points towards actual differences among traits, rather than to a statistical artifact. Contrasting with ontogenic effects, experimental conditions (e.g. greenhouse or field trials) had a strong influence on the magnitude of correlations (see below), but this could not explain the diferences observed among growth traits. Although there were more studies on diameter in the field than in greenhouse, in both cases biomass was the growth trait with the greatest proportion of significant positive correlations (59% in the field, 39% under controlled conditions), whereas diameter showed the less consistent response (19% of cases with significant positive associations in the field, and none under controlled conditions).

4.2 Apparent lack of trade-offs between growth and WUE_i

Contrary to our hypothesis, we did not find any general negative association between $\delta^{13}C$ and growth. In fact, *Gr* was either positive or non-significant for all subsets tested. This suggests that the genetic association between growth and WUE_i in trees is mainly driven by changes in photosynthetic capacity, particularly among conifers, the most represented and homogeneous group in our study. When water becomes less limiting than other environmental factors, such as radiation or temperature, and assuming that growth is at least partly carbon limited, an increase in photosynthetic capacity would confer an ecological advantage by increasing growth rates, which would in turn translate into higher WUE_i due to a maximization of the carboxylation processes per unit of water loss [11,12].

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growth [10,52,53]. However, we only found significant negative correlations for three broadleaf species (*J. regia*, *E. globulus*, and hybrid *Populus* [23,35,45]), and one conifer (*P. halepensis* [14]) Among those, only *P. halepensis* is a species particularly adapted to water-limited environments, and reportedly shows the aforementioned drought-avoiding strategy [52]. Despite the lack of statistical evidence for publication bias, a close look at the dataset suggests certain overrepresentation of highly productive species. For example, in the Mediterranean region, about 70% of the studies focused on *Pinus pinaster*, whereas the genus *Quercus* was represented in the whole dataset only by the evergreen *Q. suber*. Similarly, the genus *Larix* is only represented by *Larix occidentalis*, the largest and most productive species in the genus [54]. Unexpectedly, among the studies testing for the effect of water availability we did not find significant differences between irrigated and drought conditions, suggesting that drought treatments were either too mild or too brief as to result in a divergent response in plant $\delta^{13}C$. On the other hand, it should be noted that the species tested in these experiments were also highly productive (*P. pinaster*, *P. ponderosa*, *Eucalyptus* spp., *C. sativa*, *Pseudotsuga menziesii*, *L. occidentalis*, *Picea glauca*).

4.3 Association between WUE_i and productivity varies across biomes and functional types

The positive association between $\delta^{13}C$ and growth increased from water-limited to energy-limited environments. Whereas in the Mediterranean biome the global correlation was not significant, subtropical and temperate species showed significant positive intra-specific associations, but still of lower magnitude than in boreal species. This partly supports our initial hypothesis, indicating that genetic differences in growth potential would become more positively associated with WUE_i when moving from water-limited to energy-limited conditions. In this regard, Tang et al. [55] found a consistent increase in WUE_i rising from subtropics to mid-high latitudes (around 50-60º N), which also points toward the existence of a gradient from water-driven to radiation-driven changes in WUE_i. In line with the proposed water - radiation gradient, height was the growth trait showing the most consistent changes across biomes. This could be interpreted as a trade-off between the demand for light interception, and the increasing water and nutrient limitations with height [47,48]. Noteworthy, the non-significant trend found in the Mediterranean biome was mainly attributed to the negative effect of studies performed under suboptimal conditions, hence the most susceptible to show this trade-off (Figure 3b). The varying intra-specific correlations

between $\delta^{13}C$ and growth in water-limited environments may be a consequence of the greater environmental pressure under such conditions. Under stress, growth responses become strongly constrained by environmental factors, hence limiting the expression of genetic variability [56]. Indeed, environmental correlations between $\delta^{13}C$ and growth follow the opposite pattern along environmental gradients, showing higher correlations under water-limited conditions [57].

Unexpectedly, our meta-analysis did not reveal any significant intra-specific association between WUE_i and productivity in broadleaves, contrasting with the consistent positive correlations found in conifers and shrubs. Despite the aforementioned bias towards the most productive species, the consistent positive association between $\delta^{13}C$ and growth in conifers might be related to ancestral physiological traits, in agreement with the slower evolution rates and less dynamic radiation of gymnosperms, as compared to angiosperms [4]. Conifers in particular have highly conserved anatomical traits, from subtropical to boreal biomes, and (both deciduous and evergreen) tend to show a limited reliance on pre-stored carbohydrates for secondary growth [58]. Altogether, this is likely to result in a tighter link between leaf-level processes and whole-tree growth [15,52]. Conversely, broadleaves show large variations in terms of hydraulic conductivity and stomatal response, ranging from water-spending strategies in some ring-porous species to more isohydric responses in diffuse-porous [53]. In line with this, [55] reported contrasting environmental responses in (Mediterranean/subtropical) broadleaf evergreen and (temperate) broadleaf deciduous. Whereas broadleaf evergreens showed negative responses to radiation, and positive responses to water availability, the opposite was the case for deciduous forests. Noticeably, the only broadleaf evergreen in our study, the Mediterranean *Quercus suber*, showed a positive association between $\delta^{13}C$ and growth, contrasting with the strong negative associations found for the meso-Mediterranean *J. regia* [23]. Although not included in the analyses due to the small sample sice, other studies with deciduous oaks did not found clear associations between growth and $\delta^{13}C$ [59,60]. Overall, the complexity in the response of broadleaves cannot be definitively solved with the rather unbalanced information present in the literature, but a closer look into the underlying anatomical and/or phylogenetic traits might help to ellucidate these contrasting patterns.

4.4 Experimental conditions maximizing the relationship

between WUE_i and productivity

Ecological and/or functional inter-specific differences (e.g. across species, genera or biomes) appeared as the main drivers of between-studies heterogeneity. However, experimental conditions also exhibited a significant effect on the intra-specific association between $\delta^{13}C$ and growth. The strength of the genetic link between growth and $\delta^{13}C$ decreased with experimental upscaling, from greenhouse and growth-chamber experiments to nursery and field trials. These findings exemplify the constant dilemma for plant breeders: either maximizing genetic response under optimal, controlled conditions, or obtaining a more realistic, but noisier, evaluation in the field [5,8]. Our meta-analysis also revealed that leaf $\delta^{13}C$ (rather than wood $\delta^{13}C$) was more consistently associated with growth. Several studies have shown that the original isotopic signal imprinted in the leaves can be dampened, or even reversed, due to post-photosynthetic fractionation processses during the export and transport of assimilates from the leaves to the trunk [15]. Hence, although wood $\delta^{13}C$ offers greater time-integration, the use of leaf $\delta^{13}C$ provides a more direct insight into leaf-level processes. The interference caused by post-photosynthetic transport processes in large trees, together with the larger proportion of leaf biomass in young individuals (and overall higher whole-plant metabolic activity, see e..g [22]), could also explain the stronger correlations observed in seedlings, as compared to saplings and adult trees. Overall, our findings highlight that $\delta^{13}C$ has a tighter correlation with growth in small-scale systems (e.g. juvenile *vs.* adult, greenhouse *vs*. field). However, the strong correlations found in greenhouse experiments and seedlings may not necessarily hold in the field for adult trees (see e.g. [37]), and in this regard further research on the upscaling from seedling experiments to field trials using adult trees is needed [5,43,50].

5 Conclusions

Our meta-analysis indicated that intra-specific differences in biomass production, and to a lesser extent, height, are mainly associated to enhanced WUE_i, whereas diameter showed no significant association with WUE_i. Contrary to our hypothesis, we did not find a consistent negative growth response to increasing WUE_i under suboptimal conditions. Nevertheless, the strength of the genetic association between growth and WUE_i clearly increases from water-limited to energy-limited environments, e.g. from Mediterranean to subtropical, temperate and boreal biomes, or from suboptimal to optimal testing conditions. The bias in number of genetic studies towards the most productive species, particularly under Mediterranean conditions, may be partly responsible for the limited amount of significant negative associations reported in the literature, which contrasts with the trade-offs between growth and drought adaptations widely reported in ecological studies. Conifers show more consistent positive correlations between WUE_i and growth than broadleaves, probably linked to more conserved anatomical and physiological traits. The correlation between growth and WUE_i is maximized for seedlings evaluated under controlled and optimal conditions, and with leaves as sampling material, highlighting that the link between leaf-level physiology and growth decreases with the complexity of the system.

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6 References

[1] H.Tian, G.Chen, M.Liu, C.Zhang, G.Sun, C.Lu, X.Xu, W.Ren, S.Pan, A.Chappelka. Model estimates of net primary productivity, evapotranspiration, and water use efficiency in the terrestrial ecosystems of the southern United States during 1895-2007, For Ecol Manage. 259 (2010) 1311-1327.

[2] J.J.Landsberg, R.H.Waring. A generalised model of forest productivity using simplified concepts of radiation-use efficiency, carbon balance and partitioning, For Ecol Manage. 95 (1997) 209-228.

[3] F.J.Alberto, S.N.Aitken, R.Alía, S.C.González-Martínez, H.Hänninen, A.Kremer, F.Lefèvre, T.Lenormand, S.Yeaman, R.Whetten, O.Savolainen. Potential for evolutionary responses to climate change - evidence from tree populations, Global Change Biol. 19 (2013) 1645-1661.

[4] A.R.Leitch, I.J.Leitch. Ecological and genetic factors linked to contrasting genome dynamics in seed plants, New Phytol. 194 (2012) 629-646.

[5] T.A.Martin, K.H.Johnsen, T.L.White. Ideotype development in southern pines: rationale and strategies for overcoming scale-related obstacles, For Sci. 47 (2001) 21-28.

[6] A.K.Knapp, J.M.Briggs, J.K.Koelliker. Frequency and Extent of Water Limitation to Primary Production in a Mesic Temperate Grassland , Ecosystems. (2001) 4(1), 19–28. http://doi.org/10.2307/3658783.

[7] M.L.Roderick, P.Greve, G.D.Farquhar. On the assessment of aridity with changes in atmospheric CO2, Water Resources Research. 51 (2015) 5450-5463.

[8] I.Aranda, E.Gil-Pelegrín, A.Gascó, M.A.Guevara, J.F.Cano, M.De Miguel, J.A.Ramírez-Valiente, J.J.Peguero-Pina, P.Perdiguero, A.Soto. Drought response in forest trees: from the species to the gene, in: Plant Responses to Drought Stress, Springer, 2012, pp. 293-333.

[9] G.D.Farquhar, R.A.Richards. Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes, Aust J Plant Physiol. 11 (1984) 539-552.

[10] O.Brendel, D.Pot, C.Plomion, P.Rozenberg, J.M.Guehl. Genetic parameters and QTL analysis of delta C-13 and ring width in maritime pine, Plant Cell Environ. 25 (2002) 945-953.

[11] K.H.Johnsen, J.E.Major. Gas exchange of 20-year-old black spruce families displaying a genotype x environment interaction in growth rate, Can J For Res. 25 (1995) 430-439.

[12] Q.A.Nguyen, A.Ferhi, D.Loustau, J.M.Guehl. Within-ring delta13C spatial variability and interannual variations in wood cellulose of two contrasting provenances of Pinus pinaster, Can J For Res. 28 (1998) 766-773.

[13] E.Y.Raddad, O.Luukkanen, A.A.Salih, V.Kaarakka, M.A.Elfadl. Productivity and nutrient cycling in young Acacia senegal farming systems on Vertisol in the Blue Nile region, Sudan, Agroforest Syst. 68 (2006) 193-207.

[14] J.Voltas, M.R.Chambel, M.A.Prada, J.P.Ferrio. Climate-related variability in carbon and oxygen stable isotopes among populations of Aleppo pine grown in common-garden tests, Trees-Structure and Function. 22 (2008) 759-769.

[15] A.Gessler, J.P.Ferrio, R.Hommel, K.Treydte, R.A.Werner, R.K.Monson. Stable isotopes in tree rings: towards a mechanistic understanding of isotope fractionation and mixing processes from the leaves to the wood, Tree Physiol. 34 (2014) 796-818.

[16] C.Körner. Carbon limitation in trees, Journal of Ecology. 91 (2003) 4-17.

[17] S.Fatichi, S.Leuzinger, C.Körner. Moving beyond photosynthesis: from carbon source to sink-driven vegetation modeling, New Phytol. 201 (2014) 1086-1095.

[18] V.Resco de Dios, T.E.Mereed, J.P.Ferrio, D.T.Tissue, J.Voltas. Intra-specific variation in juvenile tree growth under elevated CO2 alone and with O3 - A meta-analysis, Tree Physiol. (2016) **doi:10.1093/treephys/tpw026**.

[19] J.W.White, J.A.Castillo, J.R.Ehleringer. Associations between productivity, root growth and carbon isotope discrimination in Phaseolus vulgaris under water deficit, Aust J Plant Physiol. 17 (1990) 189-198.

[20] J.Lynch. Root architecture and plant productivity, Plant Physiol. 109 (1995) 7.

[21] P.B.Reich, M.G.Tjoelker, J.L.Machado, J.Oleksyn. Universal scaling of respiratory metabolism, size and nitrogen in plants, Nature. 439 (2006) 457-461.

[22] S.Mori, K.Yamaji, A.Ishida, S.G.Prokushkin, O.V.Masyagina, A.Hagihara, A.T.M.Hoque, R.Suwa, A.Osawa, T.Nishizono, T.Ueda, M.Kinjo, T.Miyagi, T.Kajimoto, T.Koike, Y.Matsuura, T.Toma, O.A.Zyryanova, A.P.Abaimov, Y.Awaya, M.G.Araki, T.Kawasaki, Y.Chiba, M.Umari. Mixed-power scaling of whole-plant respiration from seedlings to giant trees, Proceedings of the National Academy of Sciences. 107 (2010) 1447-1451.

[23] N.Aletà, A.Vilanova, R.Díaz, J.Voltas. Genetic variation for carbon isotope

composition in Juglans regia L.: relationships with growth, phenology and climate of origin, Ann For Sci. 66 (2009) 1-11.

[24] K.H.Johnsen, L.B.Flanagan, D.A.Huber, J.E.Major. Genetic variation in growth, carbon isotope discrimination, and foliar N concentration in Picea mariana: analyses from a half-diallel mating design using field-grown trees, Can J For Res. 29 (1999) 1727-1735.

[25] M.Lauteri, A.Pliura, M.C.Monteverdi, E.Brugnoli, F.Villani, G.Eriksson. Genetic variation in carbon isotope discrimination in six European populations of Castanea sativa Mill. originating from contrasting localities, Journal of Evolutionary Biology. 17 (2004) 1286-1296.

[26] V.Resco de Dios, M.E.Loik, R.A.Smith, M.J.Aspinwall, D.T.Tissue. Genetic variation in circadian regulation of nocturnal stomatal conductance enhances plant fitness, Plant Cell Environ. 39 (2016) 3-11.

[27] J.Toillon, B.Rollin, E.Dallé, M.Feinard-Duranceau, J.C.Bastien, F.Brignolas, N.Marron. Variability and plasticity of productivity, water-use efficiency, and nitrogen exportation rate in Salix short rotation coppice, biomass and bioenergy. 56 (2013) 392-404.

[28] J.W.Zhang, L.Fins, J.D.Marshall. Stable carbon isotope discrimination, photosynthetic gas exchange, and growth differences among western larch families, Tree Physiol. 14 (1994) 531-539.

[29] W.Viechtbauer. Conducting meta-analyses in R with the metafor package, Journal of Statistical Software. 36 (2010) 1-48.

[30] R.A.Fisher. On the 'Probable Error' of a cocient of correlation deduced from a small sample, Metron. 1 (1921) 1-32.

[31] M.Borenstein, L.V.Hedges, J.P.T.Higgins, H.Rothstein. Introduction to Meta-Analysis, Wiley, Chichester, UK, 2009.

[32] T.B.Huedo-Medina, J.Sánchez-Meca, F.Marín-Martínez, J.Botella. Assessing heterogeneity in meta-analysis: Q statistic or I2 index?, Psychological methods. 11 (2006) 193.

[33] M.Egger, G.D.Smith, M.Schneider, C.Minder. Bias in meta-analysis detected by a simple, graphical test, Bmj. 315 (1997) 629-634.

[34] M.S.Rosenberg. The file-drawer problem revisited: a general weighted method for calculating fail-safe numbers in meta-analysis, Evolution. 59 (2005) 464-468.

[35] P.Pita, F.Soria, I.Canas, G.Toval, J.A.Pardos. Carbon isotope discrimination and its

relationship to drought resistance under field conditions in genotypes of Eucalyptus globulus Labill, For Ecol Manage. 141 (2001) 211-221.

[36] R.E.Pennington, C.R.Tischler, H.B.Johnson, H.W.Polley. Genetic variation for carbon isotope composition in honey mesquite (Prosopis glandulosa), Tree Physiol. 19 (1999) 583-589.

[37] J.W.Zhang, J.D.Marshall, L.Fins, . Correlated population differences in dry matter accumulation, allocation, and water-use efficiency in three sympatric conifer species, For Sci. 42 (1996) 242-249.

[38] K.H.Johnsen, L.B.Flanagan, D.A.Huber, J.E.Major. Genetic variation in growth, carbon isotope discrimination, and foliar N concentration in Picea mariana: analyses from a half-diallel mating design using field-grown trees, Can J For Res. 29 (1999) 1727-1735.

[39] R.D.Guy, D.L.Holowachuk. Population differences in stable carbon isotope ratio of Pinus contorta Dougl. ex Loud.: relationship to environment, climate of origin, and growth potential, Can J Bot. 79 (2001) 274-283.

[40] Z.H.Xu, P.G.Saffigna, G.D.Farquhar, J.A.Simpson, R.J.Haines, S.Walker, D.O.Osborne, D.Guinto. Carbon isotope discrimination and oxygen isotope composition in clones of the F1 hybrid between slash pine and Caribbean pine in relation to tree growth, water-use efficiency and foliar nutrient concentration, Tree Physiol. 20 (2000) 1209-1217.

[41] I.Aranda, R.Alía, U.Ortega, A.K.Dantas, J.ajada. Intra-specific variability in biomass partitioning and carbon isotopic discrimination under moderate drought stress in seedlings from four Pinus pinaster populations, Tree Genetics & Genomes. 6 (2010) 169-178.

[42] O.Roupsard, H.I.Joly, E.Dreyer. Variability of initial growth, water-use efficiency and carbon isotope discrimination in seedlings of Faidherbia albida (Del.) A. Chev., a multipurpose tree of semi-arid Africa. Provenance and drought effects. 55(3), 329-348. 1998. EDP Sciences.

[43] E.Marguerit, L.Bouffier, E.Chancerel, P.Costa, F.Lagane, J.M.Guehl, C.Plomion, O.Brendel. The genetics of water-use efficiency and its relation to growth in maritime pine, J Exp Bot. 65 (2014) 4757-4768.

[44] J.A.Ramírez-Valiente, Z.Lorenzo, A.Soto, F.Valladares, L.Gil, I.Aranda. Elucidating the role of genetic drift and natural selection in cork oak differentiation regarding drought tolerance, Molecular Ecology. 18 (2009) 3803-3815.

[45] S.Y.Dillen, N.Marron, B.Koch, R.Ceulemans. Genetic variation of stomatal traits and carbon isotope discrimination in two hybrid poplar families (Populus deltoides 'S9-2' x P. nigra 'Ghoy' and P. deltoides 'S9-2' x P. trichocarpa 'V24'), Ann Bot. 102 (2008) 399-407.

[46] S.Zhang, Q.L.Dang, X.Yü. Nutrient and [CO 2] elevation had synergistic effects on biomass production but not on biomass allocation of white birch seedlings, For Ecol Manage. 234 (2006) 238-244.

[47] M.D.Cramer. Unravelling the limits to tree height: a major role for water and nutrient trade-offs, Oecologia. 169 (2012) 61-72.

[48] T.Klein, C.Randin, C.Körner. Water availability predicts forest canopy height at the global scale, Ecol Lett. 18 (2015) 1311-1320.

[49] J.D.Bontemps, J.C.Hervé, J.F.Dhote. Dominant radial and height growth reveal comparable historical variations for common beech in north-eastern France, For Ecol Manage. 259 (2010) 1455-1463.

[50] J.D.Marshall, R.A.Monserud. Homeostatic gas-exchange parameters inferred from 13C/12C in tree rings of conifers, Oecologia. 105 (1996) 13-21.

[51] A.Duquesnay, N.Breda, M.Stievenard, J.L.Dupouey. Changes of tree-ring delta13C and water-use efficiency of beech (Fagus sylvatica L.) in north-eastern France during the past century, Plant Cell Environ. 21 (1998) 565-572.

[52] J.P.Ferrio, A.Florit, A.Vega, L.Serrano, J.Voltas. $D^{13}C$ and tree-ring width reflect different drought responses in *Quercus ilex* and *Pinus halepensis*, Oecologia. 137 (2003) 512-518.

[53] T.Klein. The variability of stomatal sensitivity to leaf water potential across tree species indicates a continuum between isohydric and anisohydric behaviours, Funct Ecol. 28 (2014) 1313-1320.

[54] W.C.Schmidt, K.J.McDonald. Ecology and management of Larix forests: a look ahead. Proceedings of an International Symposium, Whitefish, Montana, October, 5-9, 1992., USDA Forest Service, Ogden, Utah, USA, 1995.

[55] X.Tang, H.Li, A.R.Desai, Z.Nagy, J.Luo, T.E.Kolb, A.Olioso, X.Xu, L.Yao, W.Kutsch. How is water-use efficiency of terrestrial ecosystems distributed and changing on Earth?, Scientific Reports. 4 (2014).

[56] C.R.Warren, J.F.McGrath, M.A.Adams. Water availability and carbon isotope discrimination in conifers, Oecologia. 127 (2001) 476-486.

[57] J.del Castillo, J.Voltas, J.P.Ferrio. Carbon isotope discrimination, radial growth, and

NDVI share spatiotemporal responses to precipitation in Aleppo pine, Trees. 29 (2015) 223-233.

[58] G.Hoch, A.Richter, C.Körner. Non-structural carbon compounds in temperate forest trees, Plant Cell Environ. 26 (2003) 1067-1081.

[59] M.Roussel, D.Le Thiec, P.Montpied, N.Ningre, J.M.Guehl, O.Brendel. Diversity of water use efficiency among Quercus robur genotypes: contribution of related leaf traits, Ann For Sci. 66 (2009) 1-10.

[60] J.Rodríguez-Calcerrada, N.Nanos, I.Aranda. The relevance of seed size in modulating leaf physiology and early plant performance in two tree species, Trees. 25 (2011) 873-884.

Table 1. Summary of results from the mixed-effect models used to test the influence of potential moderator variables on the relationship between $\delta^{13}C$ and growth. *n*: number of studies. *QM*, test statistic for the Wald-type test of model coefficients (moderators), *p*, significance of the *QM* statistic; R^2, amount of heterogeneity accounted for by the moderators included in the model. Ht, Dm, Bm, TDM and Vol, total height, diameter, stem biomass, total dry mass and stem volume, respectively; HI, DI and BAI, annual increment in height, diameter and basal area, respectively.

Moderator	Levels	*n*	*QM*	*p*	R^2 *(%)*
Growth trait	Ht, HI, Dm, DI, BAI, Bm, TDM, Vol	176	44	<0.0001	25.8
	Height(Ht+HI), Diameter(Dm+DI & BAI), Biomass(Bm+TDM+Vol)	176	26	<0.0001	18.5
Genetic material	Populations, families, clones	176	1	0.6093	0.0
Correlation	Genetic, phenotypic	176	0	0.6907	0.0
Ontogenic stage	Seedling, sapling, adult	176	22	<0.0001	17.4
Plant Part	Wood, leaves, branchlets	176	7	0.0274	3.0
	Wood, green(leaves+branchlets)	176	7	0.0081	4.1
Life form	Broadleaf, conifer, shrub	176	8	0.0232	6.6
Leaf habit	Evergreen, deciduous	176	1	0.4538	0.7
Biome	Mediterranean, subtropical, temperate, boreal	176	25	<0.0001	19.5
Genus	*Acacia, Araucaria, Austrocedrus, Castanea, Eucalyptus, Fagus, Faidherbia, Juglans, Larix, Picea, Pinus, Populus, Prosopis, Pseudotsuga, Quercus, Salix*	176	196	<0.0001	73.7
Species	24 species (see Appendix A, Table A.1)	176	293	<0.0001	85.0
Experimental set-up	Greenhouse, nursery, field	176	12	0.0021	9.5
	Greenhouse(+nursery), field	176	12	0.0005	10.1
Water Treatment	Irrigated, drought	39	0	0.9467	0.0
Environment	Optimal, suboptimal	137	6	0.0170	6.1
Environment+Water	Optimal(+irrigated), suboptimal(+drought)	176	3	0.0926	2.4

Table 2. Association between $\delta^{13}C$ and growth depending on methodological issues: growth trait, ontogenic stage and plant part. *n*, number of studies; *Gr*, global estimated correlation coefficient; Confidence Interval, 95% confidence interval of the global estimate; *p*, level of significance of the global estimate.

Moderator	Level	*n*	*Gr* [Confidence Interval]	*p*
Growth trait	Height (Ht)	65	0.35 [0.20 , 0.50]	<0.0001
	Height increment (HI)	7	-0.33 [-0.95 , 0.29]	0.2926
	Height(Ht+HI)	72	0.28 [0.12 , 0.44]	0.0006
	Diameter (Dm)	48	0.11 [0.02 , 0.20]	0.0135
	Diameter increment (DI)	12	-0.15 [-0.67 , 0.36]	0.5601
	Basal area increment (BAI)	4	-0.57 [-1.09 , -0.05]	0.0330
	Diameter(Dm+DI+BAI)	64	0.04 [-0.12 , 0.19]	0.6484
	Stem Biomass (Bm)	35	0.69 [0.52 , 0.85]	<0.0001
	Total Dry Mass (TDM)	12	0.47 [0.04 , 0.90]	0.0306
	Volume (Vol)	5	0.64 [0.32 , 0.95]	<0.0001
	Biomass (Bm+TDM+Vol)	40	0.68 [0.54 , 0.83]	<0.0001
Ontogenic stage	Seedling	97	0.44 [0.33 , 0.56]	<0.0001
	Sapling	40	-0.07 [-0.28 , 0.14]	0.5111
	Adult	39	0.26 [0.07 , 0.45]	0.0066
Plant part	Leaves	131	0.35 [0.22 , 0.48]	<0.0001
	Branchlets	6	0.46 [-0.01 , 0.92]	0.0525
	Green(Leaves+Branchlets)	137	0.36 [0.23 , 0.48]	<0.0001
	Wood	39	0.07 [-0.01 , 0.15]	0.0754

Table 3. Association between $\delta^{13}C$ and growth depending on ecological traits: life form and biome. *n*, number of studies; *Gr*, global estimated correlation coefficient; Confidence Interval, 95% confidence interval of the global estimate; *p*, level of significance of the global estimate.

Moderator	Level	*n*	*Gr* [Confidence Interval]	*p*
Life form	Broadleaf	57	0.12 [-0.08 , 0.32]	0.2510
	Conifer	102	0.34 [0.25 , 0.44]	<0.0001
	Shrub	17	0.57 [0.06 , 1.08]	0.0289
Biome	Mediterranean	37	-0.07 [-0.32 , 0.18]	0.6005
	Subtropical	43	0.20 [0.12 , 0.27]	<0.0001
	Temperate	76	0.33 [0.20 , 0.47]	<0.0001
	Boreal	20	0.74 [0.51 , 0.98]	<0.0001

Table 4. Association between $\delta^{13}C$ and growth depending on experimental set-up and environmental conditions. *n*, number of studies; *Gr*, global estimated correlation coefficient; Confidence Interval, 95% confidence interval of the global estimate; *p*, level of significance of the global estimate.

Moderator	Level	*n*	*Gr* [Confidence Interval]	*p*
Experimental set-up	Greenhouse (Grh)	49	0.58 [0.42 , 0.74]	<0.0001
	Nursery (Nrs)	5	0.45 [0.11 , 0.78]	0.0091
	Controlled(Grh+Nrs)	54	0.56 [0.42 , 0.71]	<0.0001
	Field	122	0.17 [0.06 , 0.29]	0.0038
Environment	Irrigated	19	0.63 [0.35 , 0.90]	<0.0001
	Drought	20	0.61 [0.34 , 0.89]	<0.0001
	Optimal	88	0.28 [0.17 , 0.40]	<0.0001
	Suboptimal	49	0.03 [-0.18 , 0.25]	0.7702
	Optimal (+Irrigated)	107	0.33 [0.23 , 0.44]	<0.0001
	Suboptimal (+Drought)	69	0.18 [-0.01 , 0.37]	0.0582

Figure captions

Figure 1. Z-transformed effect size and 95% confidence intervals (error bars) of individual studies correlating carbon isotope composition ($\delta^{13}C$) and growth in woody species across different biomes and life forms. Study numbers refer to the identification given in Appendix A (Table A.1).

Figure 2. Global estimated correlation coefficient (*Gr*) and standard error of the estimate error bars) for the relationship between carbon isotope composition ($\delta^{13}C$) and different growth traits in relation to a) ontogenic stage and b) plant part used for isotope analyses. Letters denote significant differences ($P<0.05$) among levels of the moderator factor (growth trait) for each ontogenic stage (a) and plant part (b).

Figure 3. Global estimated correlation coefficient (*Gr*) and standard error of the estimate error bars) for the relationship between carbon isotope composition ($\delta^{13}C$) and growth in different biomes, in relation to a) the growth trait considered and b) environmental testing conditions (optimal+irrigated / suboptimal+drought). Letters denote significant differences ($P<0.05$) among levels of the moderator factor (biome) for each growth trait (a) and environmental conditions (b).

Figure 4. Global estimated correlation coefficient (*Gr*) and standard error of the estimate error bars) for the relationship between carbon isotope composition ($\delta^{13}C$) and growth under different environmental conditions, in relation to the experimental set-up (under controlled conditions -greenhouse and nursery- or in the field). Letters denote significant differences ($P<0.05$) among levels of the moderator factor (environment) for each experimental set-up.

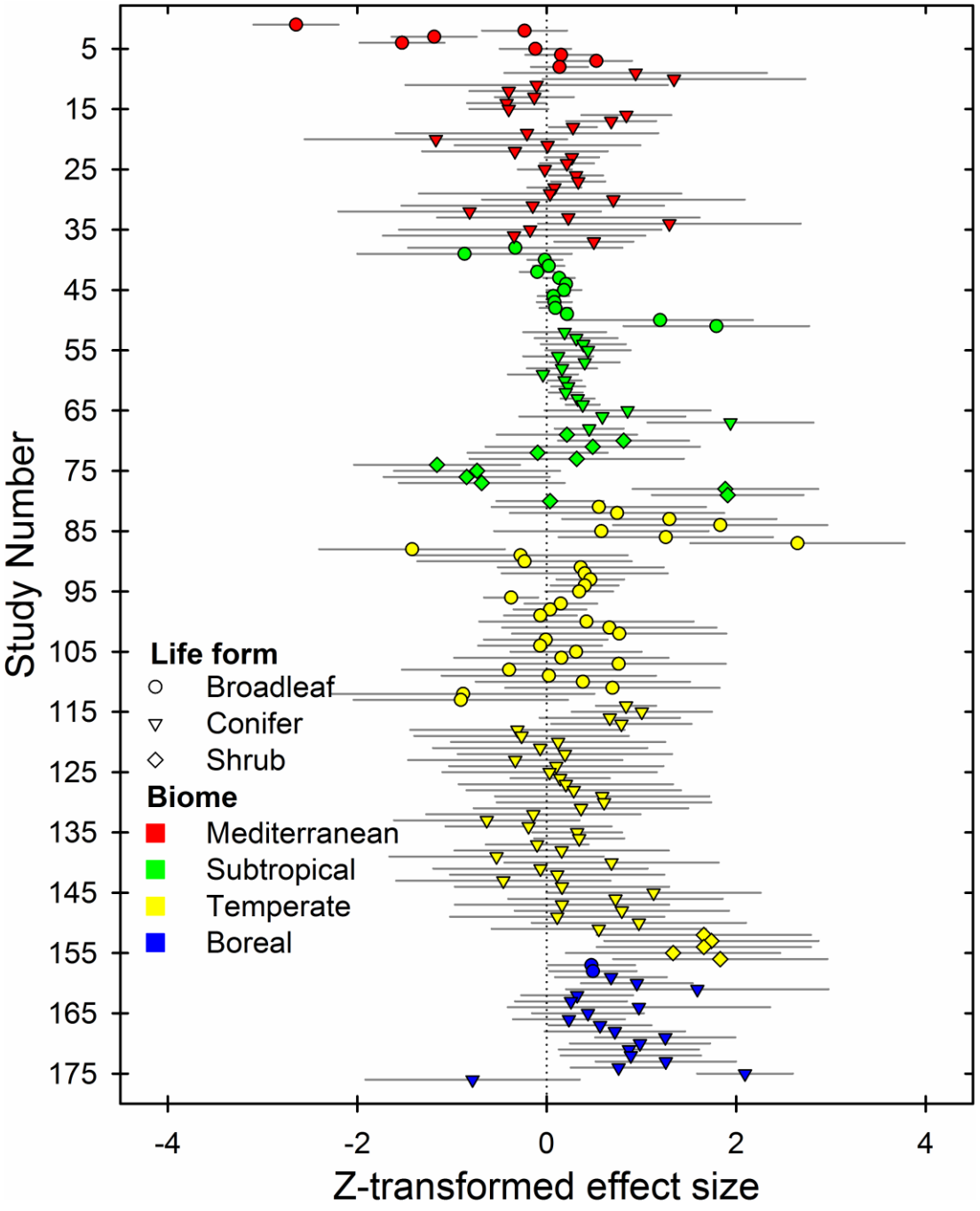
Study Number
5
15
25
35
45
55
65
75
85
95
105
115
125
135
145
155
165
175
Life form
Broadleaf
Conifer
Shrub
Biome
Mediterranean
Subtropical
Temperate
Boreal
-4
-2
0
2
4
Z-transformed effect size

Figure 1.

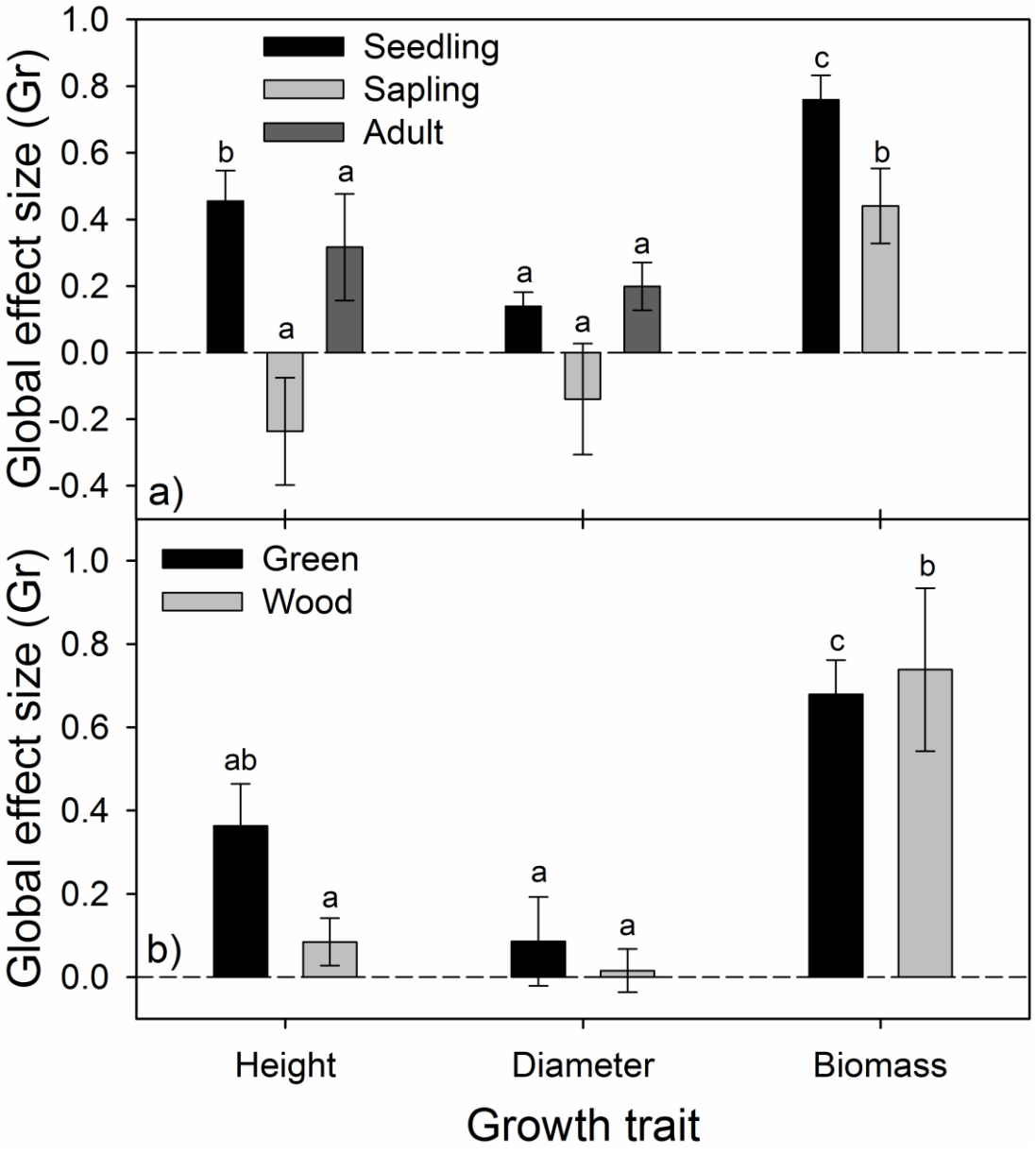
Seedling
Sapling
Adult
Green
Wood
Global effect size (Gr)
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
a)
b)
b
a
a
a
a
c
b
ab
a
a
a
c
b
Height
Diameter
Biomass
Growth trait

Figure 2.

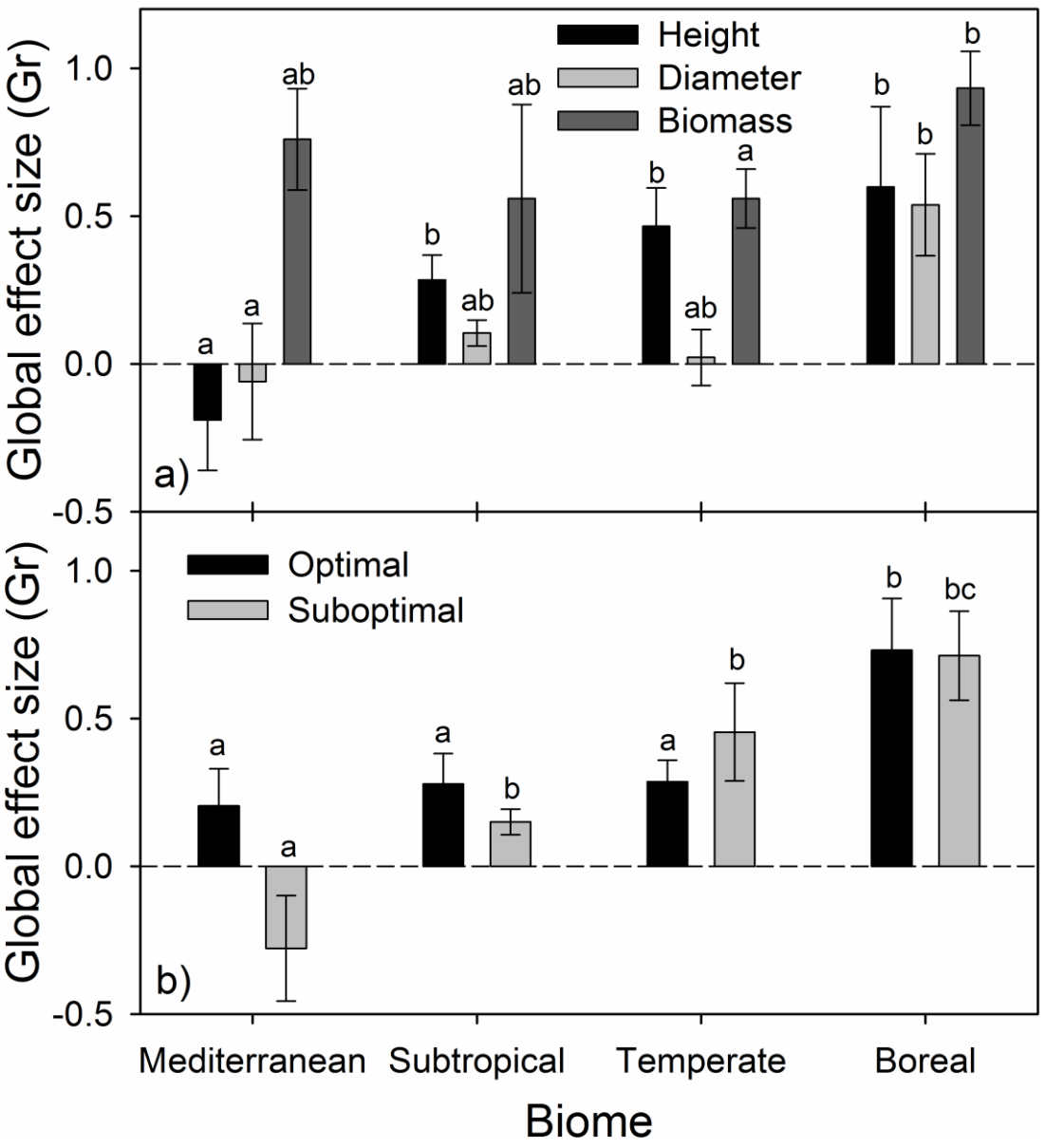
Height
Diameter
Biomass
Global effect size (Gr)
1.0
0.5
0.0
-0.5
a)
a
a
ab
b
ab
ab
b
ab
a
b
b
b
Optimal
Suboptimal
Global effect size (Gr)
b)
a
a
a
b
a
b
b
bc
Mediterranean
Subtropical
Temperate
Boreal
Biome

Figure 3.

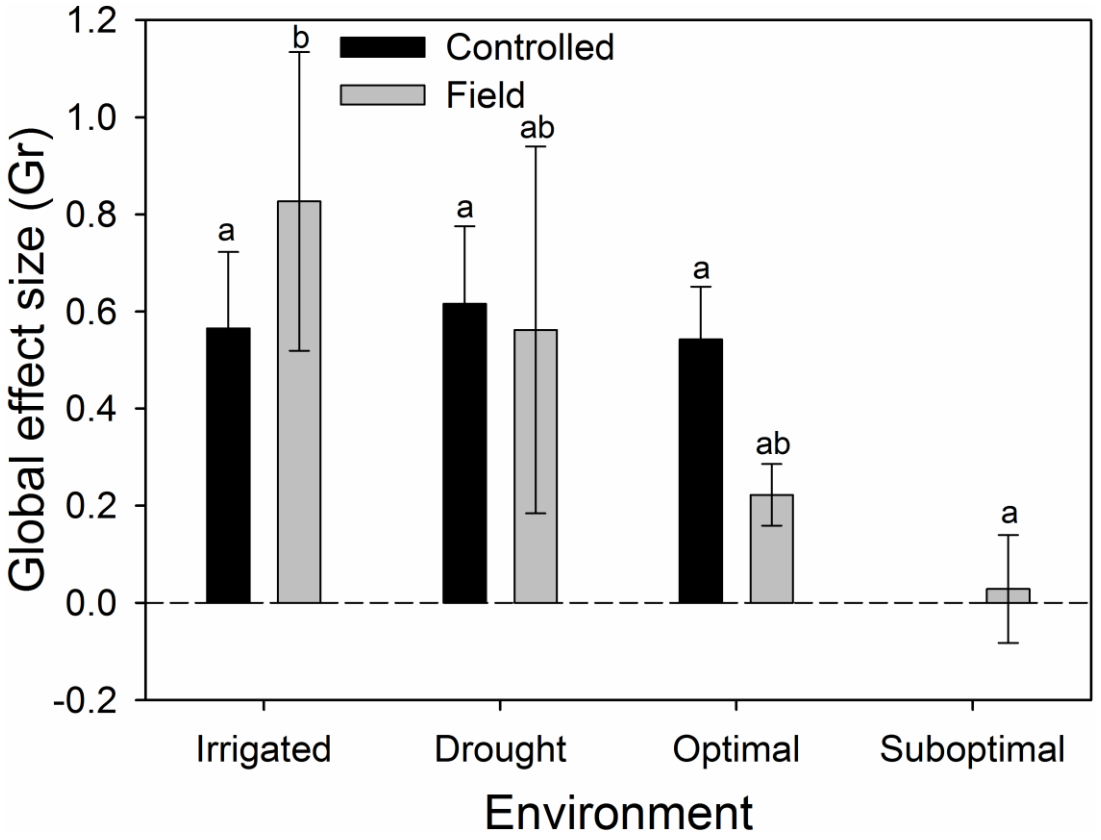
Global effect size (Gr)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
Controlled
Field
a
b
a
ab
a
ab
a
Irrigated
Drought
Optimal
Suboptimal
Environment

Figure 4.

Table 1. Summary of results from the mixed-effect models used to test the influence of potential moderator variables on the relationship between $\delta^{13}C$ and growth. *n*: number of studies. *QM*, test statistic for the Wald-type test of model coefficients (moderators), *p*, significance of the *QM* statistic; R^2, amount of heterogeneity accounted for by the moderators included in the model. Ht, Dm, Bm, TDM and Vol, total height, diameter, stem biomass, total dry mass and stem volume, respectively; HI, DI and BAI, annual increment in height, diameter and basal area, respectively.

Moderator	Levels	*n*	*QM*	*p*	R^2 *(%)*
Growth trait	Ht, HI, Dm, DI, BAI, Bm, TDM, Vol	176	44	<0.0001	25.8
	Height(Ht+HI), Diameter(Dm+DI & BAI), Biomass(Bm+TDM+Vol)	176	26	<0.0001	18.5
Genetic material	Populations, families, clones	176	1	0.6093	0.0
Correlation	Genetic, phenotypic	176	0	0.6907	0.0
Ontogenic stage	Seedling, sapling, adult	176	22	<0.0001	17.4
Plant Part	Wood, leaves, branchlets	176	7	0.0274	3.0
	Wood, green(leaves+branchlets)	176	7	0.0081	4.1
Life form	Broadleaf, conifer, shrub	176	8	0.0232	6.6
Leaf habit	Evergreen, deciduous	176	1	0.4538	0.7
Biome	Mediterranean, subtropical, temperate, boreal	176	25	<0.0001	19.5
Genus	*Acacia, Araucaria, Austrocedrus, Castanea, Eucalyptus, Fagus, Faidherbia, Juglans, Larix, Picea, Pinus, Populus, Prosopis, Pseudotsuga, Quercus, Salix*	176	196	<0.0001	73.7
Species	24 species (see Appendix A, Table A.1)	176	293	<0.0001	85.0
Experimental set-up	Greenhouse, nursery, field	176	12	0.0021	9.5
	Greenhouse(+nursery), field	176	12	0.0005	10.1
Water Treatment	Irrigated, drought	39	0	0.9467	0.0
Environment	Optimal, suboptimal	137	6	0.0170	6.1
Environment+Water	Optimal(+irrigated), suboptimal(+drought)	176	3	0.0926	2.4

Table 2. Association between $\delta^{13}C$ and growth depending on methodological issues: growth trait, ontogenic stage and plant part. *n*, number of studies; *Gr*, global estimated correlation coefficient; Confidence Interval, 95% confidence interval of the global estimate; *p*, level of significance of the global estimate.

Moderator	Level	*n*	*Gr* [Confidence Interval]	*p*
Growth trait	Height (Ht)	65	0.35 [0.20 , 0.50]	<0.0001
	Height increment (HI)	7	-0.33 [-0.95 , 0.29]	0.2926
	Height(Ht+HI)	72	0.28 [0.12 , 0.44]	0.0006
	Diameter (Dm)	48	0.11 [0.02 , 0.20]	0.0135
	Diameter increment (DI)	12	-0.15 [-0.67 , 0.36]	0.5601
	Basal area increment (BAI)	4	-0.57 [-1.09 , -0.05]	0.0330
	Diameter(Dm+DI+BAI)	64	0.04 [-0.12 , 0.19]	0.6484
	Stem Biomass (Bm)	35	0.69 [0.52 , 0.85]	<0.0001
	Total Dry Mass (TDM)	12	0.47 [0.04 , 0.90]	0.0306
	Volume (Vol)	5	0.64 [0.32 , 0.95]	<0.0001
	Biomass (Bm+TDM+Vol)	40	0.68 [0.54 , 0.83]	<0.0001
Ontogenic stage	Seedling	97	0.44 [0.33 , 0.56]	<0.0001
	Sapling	40	-0.07 [-0.28 , 0.14]	0.5111
	Adult	39	0.26 [0.07 , 0.45]	0.0066
Plant part	Leaves	131	0.35 [0.22 , 0.48]	<0.0001
	Branchlets	6	0.46 [-0.01 , 0.92]	0.0525
	Green(Leaves+Branchlets)	137	0.36 [0.23 , 0.48]	<0.0001
	Wood	39	0.07 [-0.01 , 0.15]	0.0754

Table 3. Association between $\delta^{13}C$ and growth depending on ecological traits: life form and biome. *n*, number of studies; *Gr*, global estimated correlation coefficient; Confidence Interval, 95% confidence interval of the global estimate; *p*, level of significance of the global estimate.

Moderator	Level	*n*	*Gr* [Confidence Interval]	*p*
Life form	Broadleaf	57	0.12 [-0.08 , 0.32]	0.2510
	Conifer	102	0.34 [0.25 , 0.44]	<0.0001
	Shrub	17	0.57 [0.06 , 1.08]	0.0289
Biome	Mediterranean	37	-0.07 [-0.32 , 0.18]	0.6005
	Subtropical	43	0.20 [0.12 , 0.27]	<0.0001
	Temperate	76	0.33 [0.20 , 0.47]	<0.0001
	Boreal	20	0.74 [0.51 , 0.98]	<0.0001

Table 4

Table 4. Association between $\delta^{13}C$ and growth depending on experimental set-up and environmental conditions. *n*, number of studies; *Gr*, global estimated correlation coefficient; Confidence Interval, 95% confidence interval of the global estimate; *p*, level of significance of the global estimate.

Moderator	Level	*n*	*Gr* [Confidence Interval]	*p*
Experimental set-up	Greenhouse (Grh)	49	0.58 [0.42 , 0.74]	<0.0001
	Nursery (Nrs)	5	0.45 [0.11 , 0.78]	0.0091
	Controlled(Grh+Nrs)	54	0.56 [0.42 , 0.71]	<0.0001
	Field	122	0.17 [0.06 , 0.29]	0.0038
Environment	Irrigated	19	0.63 [0.35 , 0.90]	<0.0001
	Drought	20	0.61 [0.34 , 0.89]	<0.0001
	Optimal	88	0.28 [0.17 , 0.40]	<0.0001
	Suboptimal	49	0.03 [-0.18 , 0.25]	0.7702
	Optimal (+Irrigated)	107	0.33 [0.23 , 0.44]	<0.0001
	Suboptimal (+Drought)	69	0.18 [-0.01 , 0.37]	0.0582

Figure 1

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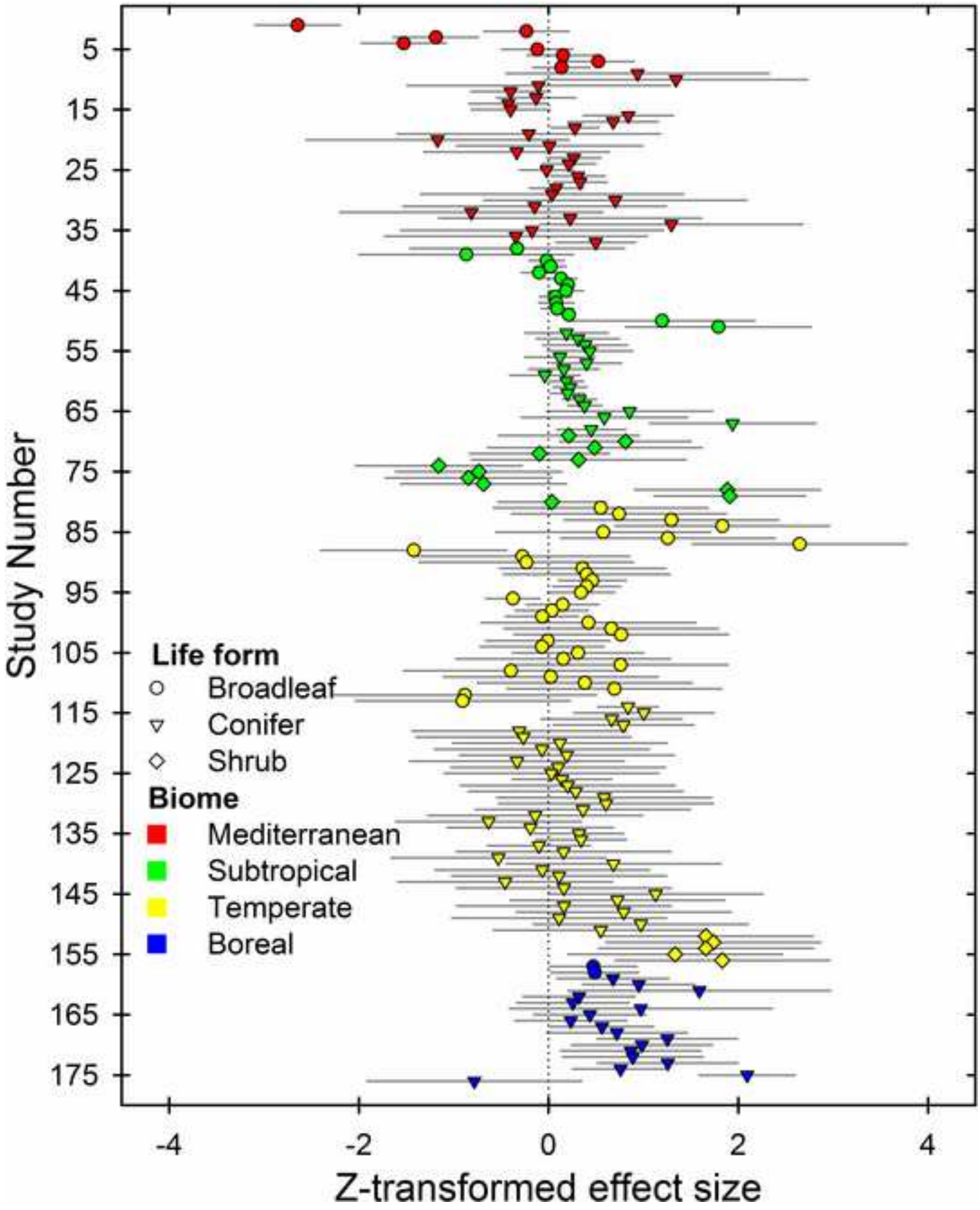

Figure 2

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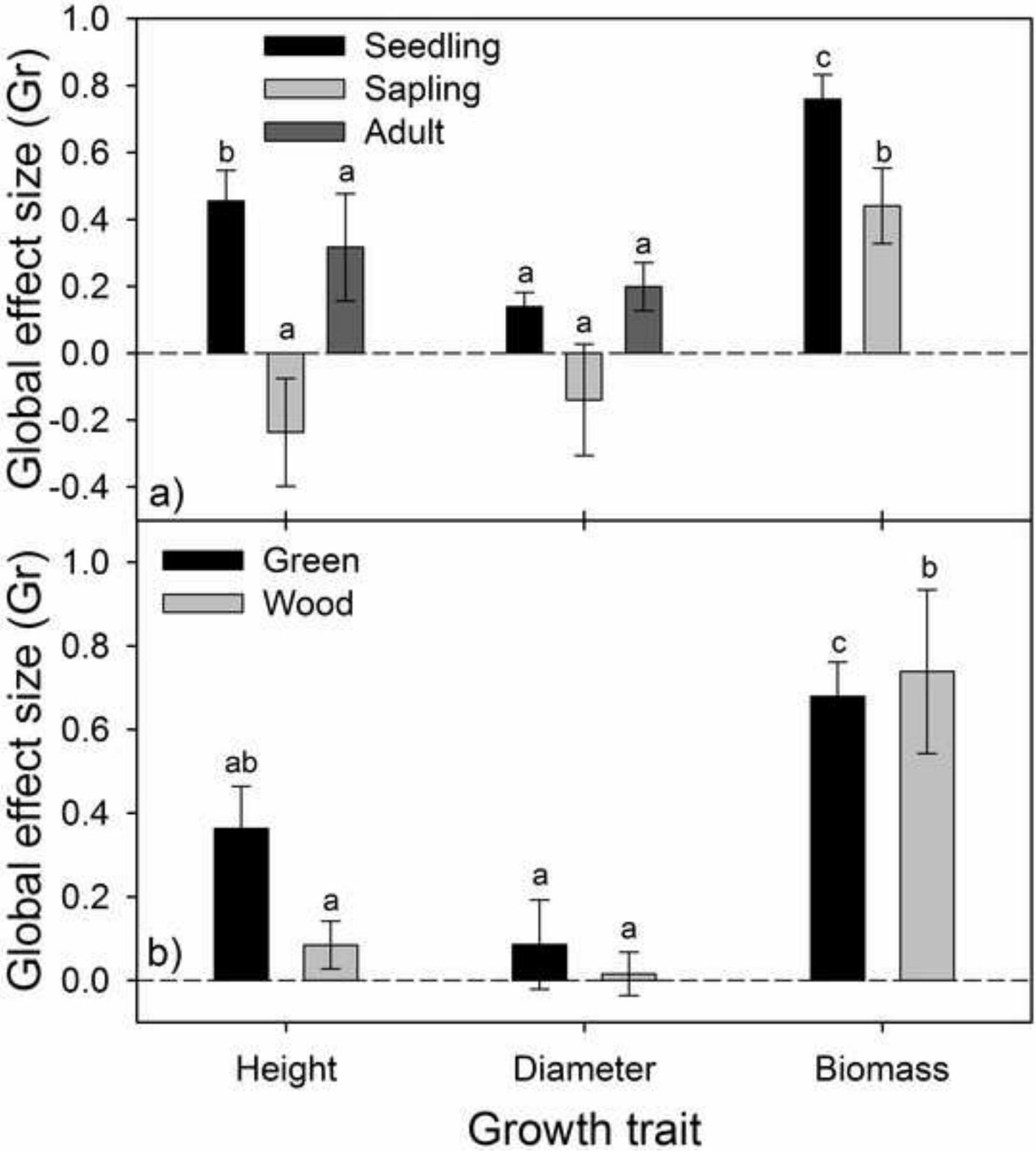

Figure 3

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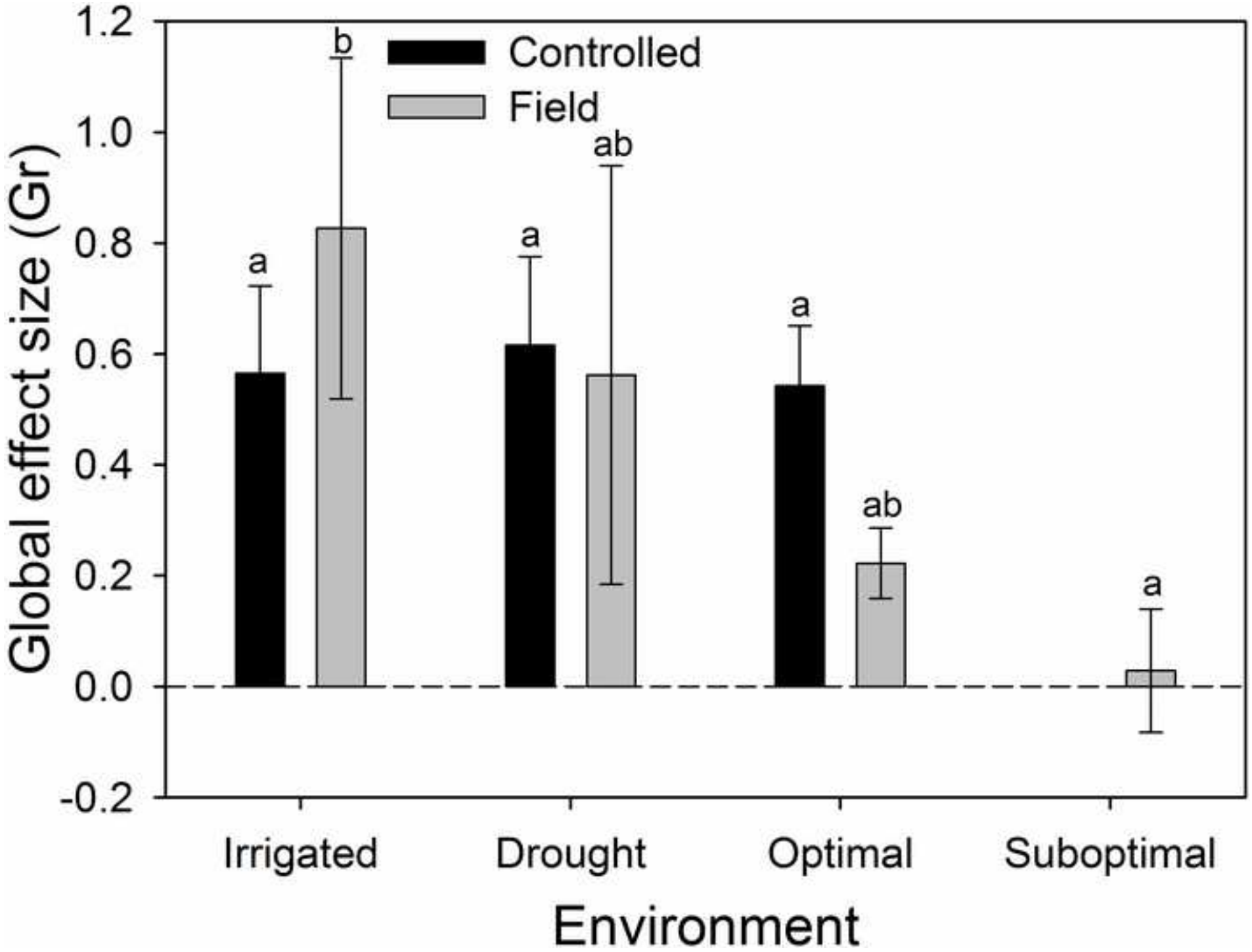

Figure 4

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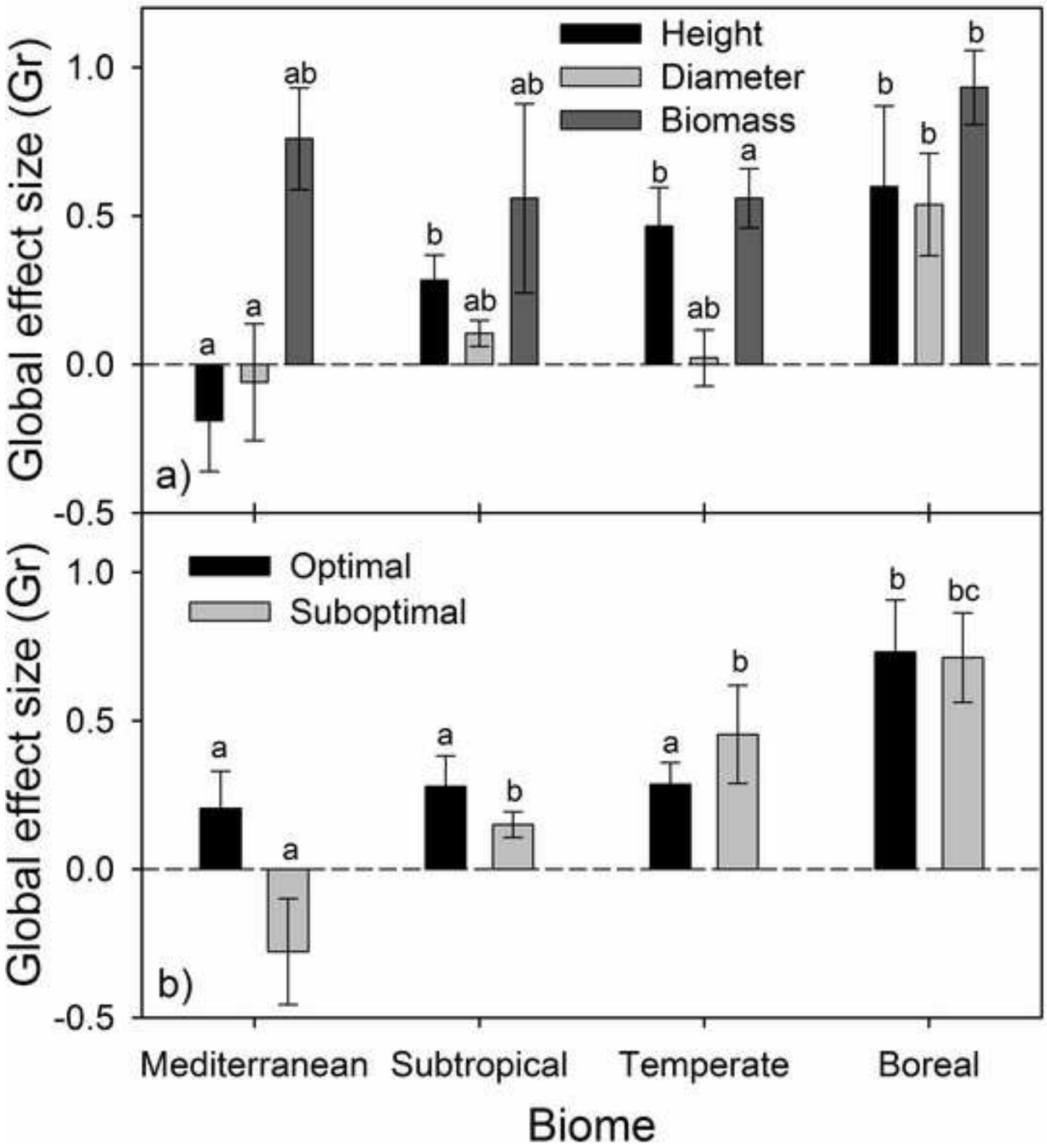

Appendix A

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